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Evaluation of the applicability and the stability of a C_{18} stationary phase containing embedded urea groups

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Abstract

Chromatographic evaluations of a new C_{18} urea phase in 150×3.9 mm HPLC columns, involving the separation of different test mixtures, indicate good performance for both polar and basic compounds when compared with a commercial C_{18} reversed phase and also show promising results for the separation of some herbicides. An aging study was performed by passing a potassium phosphate mobile phase buffered at pH 7 through 50×3.9 mm HPLC columns. The column stability was evaluated by means of the chromatographic parameters obtained for the separation of some compounds of the Neue test mixture, containing apolar, polar and highly basic analytes. The applicability of the new C_{18} urea phase was evaluated with a herbicide mixture.

Keywords: Stationary phases, LC; Urea stationary phases; Silanols; Pesticides

1. Introduction

In recent years, new alkyl silica-based stationary phases containing embedded polar groups have become increasingly popular among manufacturers and chromatographers [1]. This new bonded phase family has been developed to overcome the limitations imposed under reversed-phase conditions, especially for the separation of basic and ionizable compounds using conventional C_8 and C_{18} alkyl bonded phases.

The presence of embedded polar groups near the underlying silica surface provides superior peak shape for basic analytes regardless of the mobile phase pH, and also enhances retention in highly aqueous mobile phases, preventing phase collapse. Stationary phases with embedded amide groups were first developed using a two-step modification process where aminopropyl silica was acetylated to form the polar amide group [2–6].

Later, O'Gara and coworkers [7,8] prepared new stationary phases containing carbamate groups, based on the prior synthesis of the appropriate monofunctional chlorosilane followed by modification of the silica surface in a single-step reaction process. These carbamate phases have had their chromatographic performance compared with their alkyl counterparts and with other commercially available embedded polar stationary phases [9,10]. Engelhardt et al. [11] compared the properties of polar reversed phases from different suppliers. These authors came to the conclusion that embedded polar groups in the bonded silane not only shield the residual silanols, but also reduce the hydrophobic properties of the stationary phases, altering the overall selectivity. The potential application of this new type of stationary phase was

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also explored for the separation of phenolic compounds in wine [11].

Phases with an incorporated polar group clearly exhibit lower tailing factors for basic compounds when compared with classical bonded phases, even with phases based on high-purity silica supports. Some mechanisms have been proposed, and some evidence leads to the belief that the surface layer of an embedded polar group phase should have a higher concentration of water due to the hydrogen-bonding ability of the polar groups near the silica surface. This virtual water layer suppresses the interaction of basic analytes with the residual surface silanols [10].

Recently, we have prepared new stationary phases containing urea groups via a single-step modification process, based on the prior synthesis of new trifunctional urea-alkoxysilanes [12]. The new urea phases showed promise for the separation of the nonpolar, polar and basic compounds of the Neue test mixture with good peak shapes and column efficiencies [13].

Following publication of these phases with polar urea groups, there were questions about the stability of such phases. This study provides information on the stability of the C_{18} urea phase in a mobile phase buffered at pH 7. It is well known that silica-based stationary phases can exhibit less than desired stability in phosphate mobile phases buffered at intermediate pH [14].

The current paper also reports the separation of a mixture of herbicides (cyanazine, simazine, atrazine, ametryn, linuron and diuron) using the C_{18} urea column to better illustrate the benefits of having polar urea groups in the stationary phase. The same separations were also performed on a conventional C_{18} reversed phase to show that the new stationary phase has advantages for the separation of both neutral, polar and basic compounds.

2. Experimental

2.1. Chemicals and packings

Uracil, acetophenone, naphthalene, propranolol, butylparabene, dibutyl phthalate, acenaphthene and amitriptyline were obtained from Aldrich (Milwaukee, WI, USA) and were used as received. Cyanazine, simazine, atrazine and ametryn were kindly supplied by Norvatis. Diuron and linuron were supplied by DuPont and Hoechst, respectively. Potassium salts (KH_2PO_4 and K_2HPO_4) were purchased from Merck (Darmstadt, Germany). All other solvents (methanol and acetonitrile) were HPLC grade and were also purchased from Merck. Deionized water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). A 150×3.9 mm Nova-Pak C_{18} column (spherical octadecylsilated silica particles of 4 μm, pore size 6 nm) was purchased from Waters (Milford, MA, USA). C_{18} spherical Rainin silica (spherical, 5 μm, pore size 10 nm) was purchased from Varian (Palo Alto, CA, USA) and was slurry packed in a 150×3.9 mm HPLC column [15].

2.2. C_{18} Urea phase synthesis

ProntoSil spherical silica (3 μm), from Bischoff Chromatography (Leonberg, Germany), was chemically modified with the trifunctional urea-containing alkoxysilane $(CH_3CH_2O)_3$–Si–$(CH_2)_3$–NH–C(O)–NH–$(CH_2)_{17}$–CH_3 to obtain the C_{18} urea phase with a surface coverage of 3.22 μmol m^{-2}. Additional details about the preparation, column packing, and physicochemical and chromatographic characterization of this phase have been published previously [13].

2.3. Chromatographic separations

The chromatographic tests were performed using a modular HPLC system with a Waters 486 tuneable wavelength absorbance detector (Waters), a Waters 510 pump and a Rheodyne 7725 injector (Cotati, CA, USA). Data were processed using ChromPerfect software (Justice Innovations, Mountain View, CA, USA). Experiments were carried out at 298 K, with detection at 254 nm and an injection volume of 5 μl. All solvents were filtered and degassed before use. The mobile phases were prepared volumetrically from individually measured amounts of each component. The Neue test mixture [16] was chosen to evaluate the ability of the urea groups to reduce tailing for basic compounds and contained a mixture of uracil (12 mg l^{-1}), naphthalene (70 mg l^{-1}), acenaphthene (200 mg l^{-1}), butylparabene (26 mg l^{-1}), dibutyl phthalate (420 mg l^{-1}), propranolol

(400 mg l^{-1}), and amitriptyline (110 mg l^{-1}). Methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 (65:35, v/v) at pH 7 was used as the mobile phase. The buffer was prepared by dissolving 1.68 g K_2HPO_4 and 1.33 g KH_2PO_4 in a 1 l volumetric flask. The pH was adjusted to 7.0 using a calibrated pH meter before addition of methanol. Plate number, *N*, retention factor, *k*, and tailing factor at 5%, T_f, were calculated for column performance evaluation [17].

Separation of a mixture of cyanazine (1 mg l^{-1}), simazine (1 mg l^{-1}), atrazine (1 mg l^{-1}), ametryn (1 mg l^{-1}), linuron (2 mg l^{-1}) and diuron (2 mg l^{-1}) was performed using methanol–water (50:50, v/v) as mobile phase with detection at 230 nm.

2.4. Column aging study

For the column aging test, a small column of 50×3.9 mm was packed with the C_{18} urea phase. To simulate usual chromatographic practice, the column was continuously purged at 1.0 ml min^{-1} with methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 buffer (65:35, v/v) at pH 7.0 (not recycled) as mobile phase at ambient temperature. The column was periodically tested by the separation of uracil, naphthalene, dibutyl phthalate, propranolol, and amitriptyline at the optimal flow-rate of 0.4 ml min^{-1}. Not all components of the Neue test mixture were used for this test, because they were not well separated due to the short length of the column employed for this study.

3. Results and discussion

3.1. Properties of the C_{18} urea column

The ability of the urea groups to minimize undesirable interactions with unwanted silanol groups on the silica surface was evaluated using a test mixture containing nonpolar, polar and highly basic compounds. The test mixture, proposed by Neue [16], is composed of uracil as a marker for column dead volume, naphthalene and acenaphthene as hydrophobic markers, butylparabene and dibutyl phthalate as polar probes and propranolol and amitriptyline as basic probes. The most interesting probes in this mixture are propranolol and amitriptyline, because these compounds are highly basic ($pK_a > 9$). With a mobile phase at pH 7, the great majority of the residual surface silanols are in their ionized form ($Si–O^-$) and the basic probes are protonated (BH^+). The protonated bases interact with the deprotonated silanols by an ionic interaction, causing tailing. For this reason, the tailing and the retention factor of these two basic probes are a good measure of the silanophilic activity [9,10].

The advantage of using the C_{18} urea stationary phase for the separation of propranolol and amitriptyline is clearly observed by the striking differences in the separation, under the same conditions, on the new C_{18} urea phase and a column packed with a commercial C_{18} Rainin phase, as shown in Fig. 1. Propranolol was well separated from the other compounds with good peak shape ($T_f = 1.6$) on the C_{18} urea phase. The same behavior was not observed

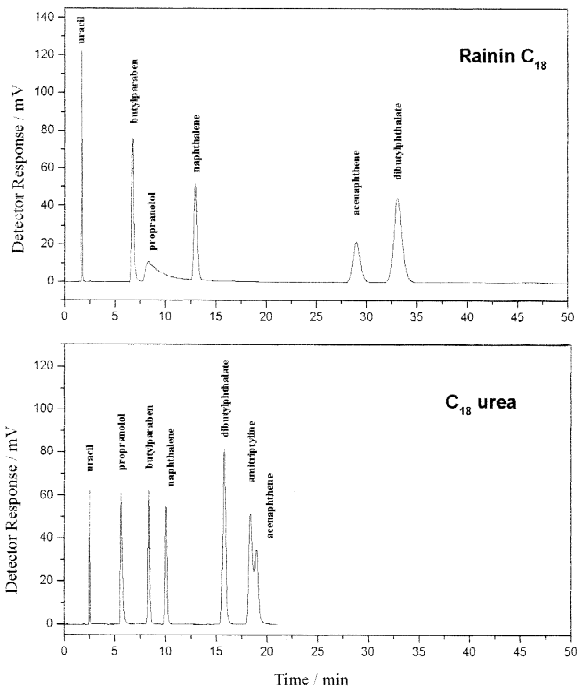

Fig. 1. Separation of the Neue test mixture composed of uracil, propranolol, butylparabene, naphthalene, dibutyl phthalate, acenaphthene and amitriptyline on the C_{18} urea and on the Rainin C_{18} phases. Conditions: 150×3.9 mm I.D. columns; mobile phase, methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (65:35, v/v) at 0.6 ml min^{-1}; detection, UV at 254 nm; injection volume, 5 μl.

Table 1
Chromatographic parameters[a] obtained for the separation of a test mixture composed of nonpolar, polar and basic analytes on the C_{18} urea column and on a C_{18} Rainin phase

Compound	C_{18} urea			Rainin C_{18}		
	k	N/m	T_f	k	N/m	T_f
Propranolol	1.25	24 530	1.60	3.96	1240	4.44
Butylparabene	2.35	79 550	1.15	3.01	34 650	1.34
Naphthalene	3.04	96 620	1.14	6.75	54 180	1.15
Dibutyl phthalate	5.35	87 850	1.06	18.8	51 590	1.13
Acenaphthene[b]	6.64	75 310*	1.12*	16.34	52 880	1.13
Amitriptyline	6.39	51 490	1.70	nd	nd	nd

nd, not detected.
[a] Chromatographic conditions: 150×3.9 mm I.D. columns packed with C_{18} urea and C_{18} Rainin phases; mobile phase, methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4 at pH 7 (65:35, v/v); optimal flow-rate, 0.6 ml min^{-1}; detection, UV at 254 nm; injection volume, 5 μl.
[b] Parameters calculated from the separate injection of acenaphthene.

on the C_{18} Rainin column. The excessive tailing and longer retention for propranolol is a clear indication of greater silanophilic activity on the C_{18} Rainin silica. With the C_{18} urea phase, amitriptyline and acenaphthene co-eluted. On the other hand, the tricyclic antidepressant was strongly retained on the conventional phase and did not elute under the conditions used in the test. Such behavior is further evidence for the strong silanophilic interactions on the C_{18} Rainin phase, whose silica support (type A) is not as high purity as the ProntoSil silica (type B) used to prepare the C_{18} urea phase and does not contain polar groups to minimize the interactions with the acidic residual surface silanols.

The chromatographic parameters were calculated for each component and are summarized in Table 1. According to these values, amitriptyline on the C_{18} urea column shows fairly good efficiency and peak shape. The tailing factor value of 1.7 is smaller, when compared with the values reported for amitriptyline, under the same separation conditions using C_{18} reversed phases, even those based on high-purity silica supports [10].

3.2. Separation of herbicides

The experiment was based on the separation of some important herbicides due to their potential for groundwater contamination. The structure of each compound is shown in Fig. 2. Fig. 3 shows the

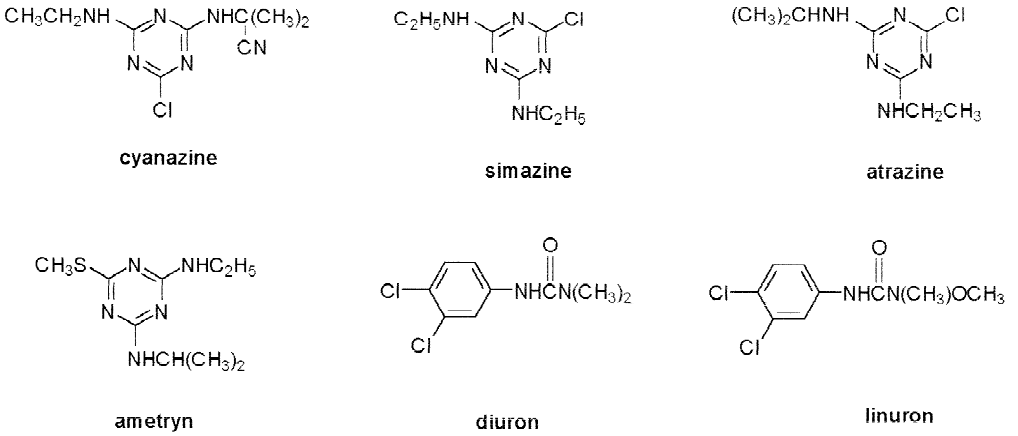

Fig. 2. Structures of the herbicides used in this study.

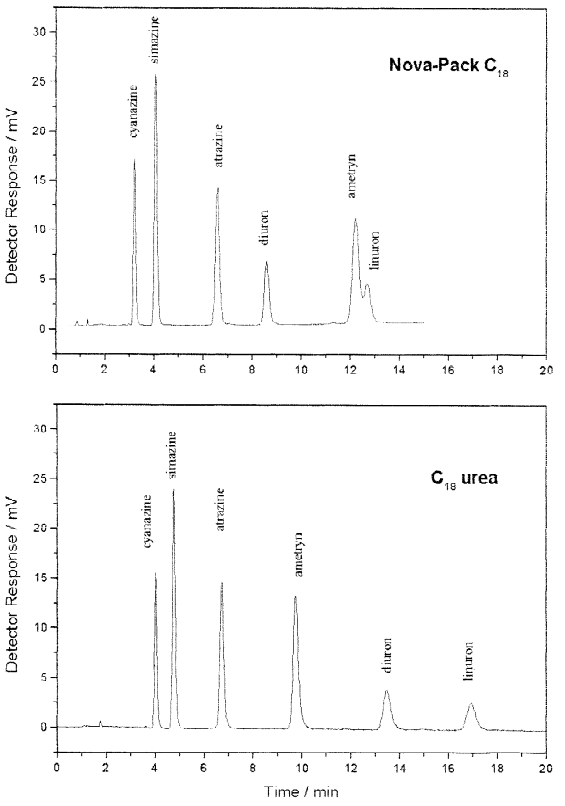

Fig. 3. Chromatograms of the separation of the mixture of herbicides composed of cyanazine, simazine, atrazine, ametryn, linuron and diuron performed on the C_{18} urea and the Nova-Pak C_{18} phases. Conditions: 150×3.9 mm I.D. columns; mobile phase, methanol–water (50:50, v/v); flow-rate, 0.8 ml min^{-1}; detection, UV at 230 nm; injection volume, 5 μl.

separations obtained on the C_{18} urea column and also on a C_{18} Nova-Pak column under the same separation conditions. The advantage of using the C_{18} urea phase over the commercial C_{18} Nova-Pak is shown by the different elution order obtained. With the commercial phase, ametryn and linuron co-eluted, while a different selectivity is observed for the phase containing the polar urea groups.

According to the structure of ametryn (shown in Fig. 2) the presence of the $-SCH_3$ substituent makes this molecule more basic than the other triazines [18]. On the urea phase, ametryn is less retained with good peak shape when compared to the conventional C_{18} reversed phase. The chromatographic parameters were calculated and are listed in Table 2. N/m values ranged from 55 800 for cyanazine to 68 300 for the most retained compound linuron. These values are relatively higher when compared with those obtained with the Nova-Pak column. The higher retention and the less than desirable tailing factor for ametryn for the conventional phase may be attributed to interactions with residual surface silanols. To overcome the co-elution of ametryn and linuron, the pH of the mobile phase can be adjusted to 4 with phosphoric acid. This minimizes the tailing and lowers retention with the conventional C_{18} phase.

Table 2
Chromatographic parameters[a] obtained for the separation of the mixture of herbicides

	C_{18} urea			Nova-Pak C_{18}		
	k	N/m	T_f	k	N/m	T_f
Cyanazine	1.27	55 770	1.20	1.50	38 570	1.15
Simazine	1.69	59 180	1.12	2.17	43 820	1.10
Atrazine	2.80	60 690	1.17	4.14	49 040	1.11
Ametryn	4.50	67 920	1.31	8.54	58 300	1.62[b]
Diuron	6.61	60 490	1.27	5.73	58 490	0.97
Linuron	8.56	68 320	1.24	8.95	46 290	1.32

[a] Chromatographic conditions: 150×3.9 mm I.D. columns packed with C_{18} urea phase and Nova-Pak C_{18}; mobile phase, methanol–water (50:50, v/v); flow-rate, 0.8 ml min^{-1}; detection, UV at 230 nm; injection volume, 5 μl.

[b] This value for the compound was obtained by individual injection.

The results obtained show the potential application of the new C_{18} urea stationary phase for the determination of these herbicides, without requiring pH adjustment of the mobile phase. This may increase the column lifetime.

3.3. Column stability

Initially, a HPLC column packed with the C_{18} urea phase was chosen for the test. There are some advantages of using a shorter column length, such as the smaller quantity of stationary phase required and the shorter analysis time. After the initial chromatographic tests, the column was purged with a known volume of the mobile phase at room temperature, periodically testing with the same test mixture. The mobile phase chosen was a mixture of methanol–20 mmol l^{-1} phosphate buffer at pH 7.0 (65:35, v/v). This system was selected as a critical test, since phosphate buffers are by far much more aggressive in the dissolution of the silica support when com-

pared with other buffers, for example citrate, Tris, etc. [19,20]. The chromatographic parameters plate number expressed as N/m, tailing factor at 5% and retention factor for naphthalene (nonpolar probe), dibutyl phthalate (polar), amitriptyline and propranolol (basic analytes) were used to monitor column stability.

The retention of naphthalene during the aging test is a good measure of stationary phase hydrophobicity. The retention factors for amitriptyline and propranolol are known to be very influenced by the presence of residual silanols at pH 7, and thus measure the silanol population on the silica surface during the aging test.

According to Fig. 4, the retention factors for naphthalene and dibutyl phthalate change less than those for the basic probes, amitriptyline and propranolol. The slight increase in the retention factors for both basic probes can be interpreted as an increase in the number of residual surface silanols after the passage of approximately 20 000 column volumes.

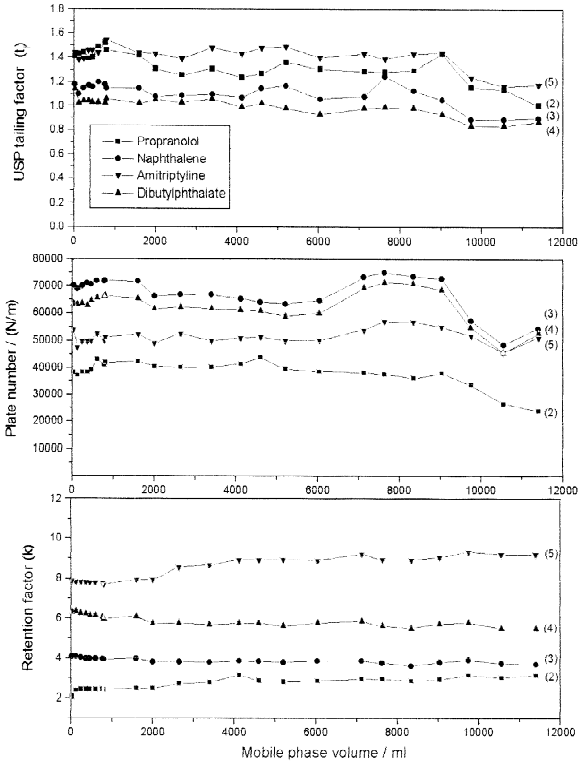

Fig. 4. Chromatographic parameters calculated during the aging test at pH 7 for the C_{18} urea stationary phase. Column, 50×3.9 mm I.D. Aging, methanol–20 mmol l^{-1} KH_2PO_4/K_2HPO_4, pH 7.0 (65:35, v/v) at 1.0 ml min^{-1}; temperature, 25 °C. Chromatographic test: uracil (1), propranolol (2), naphthalene (3), dibutyl phthalate (4) and amitriptyline (5), same mobile phase, flow-rate 0.4 ml min^{-1}, detection at 254 nm and injection volume 5 μl.

However, the retention factor is not an appropriate chromatographic parameter to evaluate stationary phase degradation. N/m values decreased for almost all compounds after the passage of 16 000 column volumes, as also shown in Fig. 4. The tailing factors also decreased at this same volume of mobile phase. The fronting effect and the decrease in the N/m values suggest a partial dissolution of the silica support by the continuous purge of the buffered mobile phase. To better illustrate the column degradation, Fig. 5 shows the chromatograms before and after the passage of 20 000 column volumes of the buffered mobile phase, when the test was arbitrarily stopped. At this point, propranolol and naphthalene are no longer well separated and dibutyl phthalate is less retained, while amitriptyline is more retained. The fronting observed for these two latter components of the test mixture is clearly shown, reinforcing the suggestion of dissolution of the silica support.

Concerns about the chemical stability of alkyl bonded silane containing a polar urea group are now resolved, since the failure of the stationary phase is mainly attributed to dissolution of the silica support and not to hydrolysis of the urea groups. It can be speculated that, if bond breakage had occurred, changes in the retention time for naphthalene would be much more representative and the tailing factors for the basic probes would also be higher. Elemental analyses of the packing material after the stability test, conversely, showed greater carbon contents when compared with the initial carbon percentage of 13%. A higher carbon percentage for the packing material at the column inlet was also observed when compared with the carbon percentage of the material near the column outlet. For the packing material removed from the inlet, a pale yellow color was observed, different from the white color of the packing material removed from the column outlet. The higher carbon percentages are another predictable as a consequence of dissolution of the silica support.

The results obtained with the column prepared in

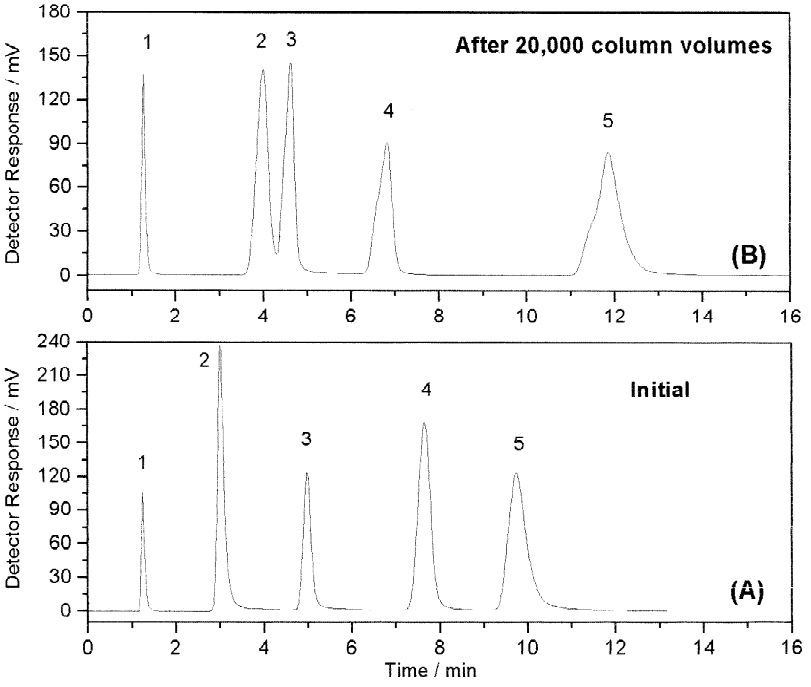

Fig. 5. Chromatograms obtained for the separation of uracil (1), propranolol (2), naphthalene (3), dibutyl phthalate (4) and amitriptyline (5) before (A) and after (B) the passage of approximately 12 000 ml of buffered mobile phase at pH 7. Chromatographic conditions are the same as in Fig. 4.

our laboratory are fairly good. Comparisons with other degradation studies for conventional reversed phases using phosphate-buffered mobile phases are difficult, because the tests were performed under more aggressive conditions, using a higher concentration of the buffer in the mobile phase and temperatures above the ambient temperature. In a systematic study [19], a stationary phase containing amide groups (Supelcosil ABZ+) was evaluated. This column was significantly degraded after the passage of 2000 column volumes of an acetonitrile–0.25 mol l^{-1} sodium phosphate buffer, pH 7 (20:80, v/v), as mobile phase at 60 °C in the separation of a mixture of tricyclic antidepressants, including amitriptyline as probe [19].

Knowing that stationary phases with embedded polar groups may have a water layer tightly bonded near the underlying silica surface due to the hydrogen-bonding ability of the polar group, it can be expected that the silica surface is more exposed to interactions with phosphate anions. As a consequence, such behavior can facilitate the hydrolysis of the siloxane bonds and enhance silica solubility. For practical purposes, to enhance the lifetime of stationary phases containing embedded polar groups, the use of phosphate buffers in mobile phases should be avoided.

4. Conclusion

The shielding effects caused by the presence of polar urea groups embedded in the C_{18} urea phase are clearly observed by the improved peak shapes obtained for the separation of the Neue test mixture at pH 7.0, a separation which was not observed with a commercial Rainin C_{18} phase. The separation of some herbicides, at neutral pH, shows the potential application of this new kind of polar stationary phase in determinations of these substances. The column aging test has shown that degradation of the column was exclusively caused by dissolution of the silica support and not by hydrolysis of the polar urea groups.

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